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ESSENTIAL GUIDES FOR ISOLATION/ PURIFICATION OF POLYSACCHARIDES

R.-C. Sun and J. Tomkinson,
University College of Wales, Bangor, UK

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Introduction

The isolation of polysaccharides from biological sources represents an important source of these valuable materials. Biomass such as cereal straws and grasses, are an enormous underutilized energy resource as raw materials in the production of paper, panel products, chemicals and other industrial products. On a dry-weight basis, the straws and grasses contain 65–85% of polysaccharides, with hemicelluloses ranked second to cellulose in abundance; however, it must be noted that the chemical content of the hemicellulose, with respect to saccharide ratios changes with plant growth and maturity (**Table 1**).

At the present time, there is widespread interest in the use of hemicelluloses, particularly arabinoxylan-rich hemicelluloses as precursors in food gums. More recently, water-soluble xylans from corn cobs have shown biological activity as immuno-modulating compounds. Other potential industrial applications of hemicelluloses are to be found in the fields of adhesives, thickeners in foods, stabilizers, biodegradable film formers and emulsifiers. They can also be easily converted to primary chemicals such as xylose, xylitol, furfural, hydroxymethylfurfural and levulinic acid.

Hemicelluloses, however, are the most complex components in the cell wall of straws and grasses. They form hydrogen bonds with cellulose, covalent bonds (mainly α-benzyl ether linkages) with lignins and ester linkages with acetyl units and hydroxycinnamic acids. To investigate the potential utilizations of polysaccharides from straws and grasses, a thorough study of the isolation procedures is necessary. Details of the method are reviewed as follows.

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Cell Wall Preparation

The straw or grass is cut into 1–2-cm lengths, air-dried, and ground to pass through a 0.5–0.8-mm screen. The ground sample is then further dried in a cabinet oven with air circulation at 50–60°C for 12–16 h. Dried material is dewaxed by refluxing with toluene–EtOH (2 : 1, v/v) or defatted with chloroform–methanol (2 : 1, v/v) for 6 h in a Soxhlet apparatus. The dewaxed sample is treated with α-amylase to degrade the starch or extracted with phenol–acetic

Table 1 Chemical composition of agricultural residues (per cent dry matter)

Species	*Water-solubles*	*Cellulose*	*Hemicelluloses*	*Lignin*	*Extract*	*Ash*
Wheat straw	4.7	38.6	32.6	14.1	1.7	5.9
Rice straw	6.1	36.5	27.7	12.3	3.8	13.3
Rye straw	4.1	37.9	32.8	17.6	2.0	3.0
Barley straw	6.8	34.8	27.9	14.6	1.9	5.7
Oat straw	4.6	38.5	31.7	16.8	2.2	6.1
Maize stems	5.6	38.5	28.0	15.0	3.6	4.2
Corn cobs	4.2	43.2	31.8	14.6	3.9	2.2
Esparto	6.1	35.8	28.7	17.8	3.4	6.5
Sugar beet pulp	(pectin 27.1) 5.9	18.4	14.8	5.9	1.4	3.7
Bagasse	4.0	39.2	28.7	19.4	1.6	5.1
Oil palm fibre	5.0	40.2	32.1	18.7	0.5	3.4
Abaca fibre	3.7	60.4	20.8	12.4	0.8	2.5

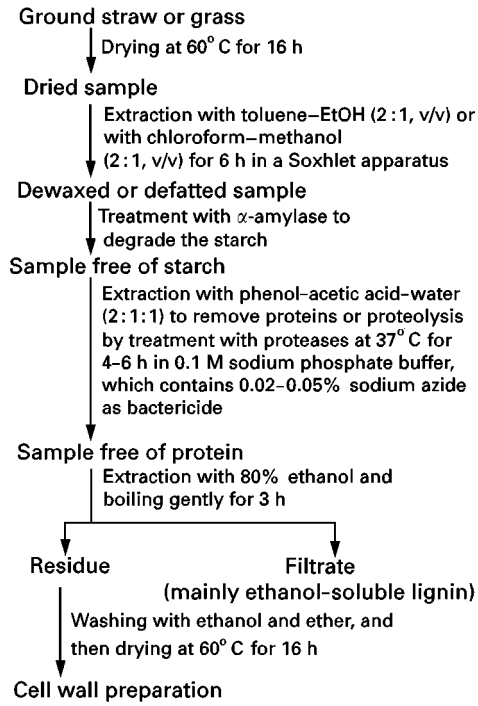
Ground straw or grass
Drying at 60° C for 16 h
Dried sample
Extraction with toluene–EtOH (2 : 1, v/v) or with chloroform–methanol (2 : 1, v/v) for 6 h in a Soxhlet apparatus
Dewaxed or defatted sample
Treatment with α-amylase to degrade the starch
Sample free of starch
Extraction with phenol–acetic acid–water (2 : 1 : 1) to remove proteins or proteolysis by treatment with proteases at 37° C for 4–6 h in 0.1 M sodium phosphate buffer, which contains 0.02–0.05% sodium azide as bactericide
Sample free of protein
Extraction with 80% ethanol and boiling gently for 3 h
Residue
Filtrate
(mainly ethanol-soluble lignin)
Washing with ethanol and ether, and then drying at 60° C for 16 h
Cell wall preparation

Figure 1 Scheme for procedures in cell wall preparation from straw or grass.

acid–water (2 : 1 : 1) to remove proteins. The proteins can also be extracted from the residue with sodium dodecyl sulfate solution containing 10 mM 1,4-dithiothreitol at room temperature for 3 h. To degrade the proteins, proteolysis is started by addition of proteases at 37°C for 4–6 h in 0.1 M sodium phosphate buffer, pH 7.5, containing 0.02–0.05% sodium azide as bactericide. The cell walls are recovered by filtration, extensively washed with water, and then treated with 80% ethanol to release the ethanol–water-soluble components. These consist mainly of free phenols and ethanol-soluble lignins together with small amounts of low-molecular-weight polysaccharides (~ 10% w/w of ethanol extract). After filtration, the cell wall preparations are dried by solvent exchange through ethanol and diethyl ether. **Figure 1** summarizes the procedures for cell wall preparation.

Fractional Extraction of Cell Wall Polysaccharides

Water-Soluble Polysaccharides

The scheme for fractional extraction of cell wall polysaccharides is illustrated in **Figure 2**. The prepared cell walls are stirred with distilled water at 75–80°C for 2 h. The residue is filtered off on a nylon cloth, washed with ethanol and ether, and dried in an oven at 60°C for 16 h. Water-soluble polysaccharides are isolated by precipitation of the concentrated supernatant with 4 volumes of 95% ethanol and recovered by centrifugation. The resultant solid is subsequently purified by extensive washing with 75% ethanol and then freeze-dried. As shown in Table 1, water-soluble polysaccharides obtained from various straws and grasses account for 3.7–6.8% of the dry matter and have much lower weight-average molecular weights ($\bar{M}_w = \sim 8000\ g\ mol^{-1}$) when compared to those of hemicellulosic fractions. Neutral sugar analysis shows that arabinose and galactose are the major sugar constituents, whereas xylose and glucose are present in only small amounts in this fraction. Detailed studies of water-soluble polysaccharides from rapeseed meal, sorghum stalk, and dehulled legume seeds and their hulls have been reported.

Pectic Polysaccharides

The pectic polysaccharides are restricted to the primary cell wall and middle lamella of higher plant tissues and growth zones. They are most abundant in soft tissues, such as rinds of citrus fruit (~30%), sugar beet pulp (~25%), and apple peels (~15%), but are present in only small proportions in woody

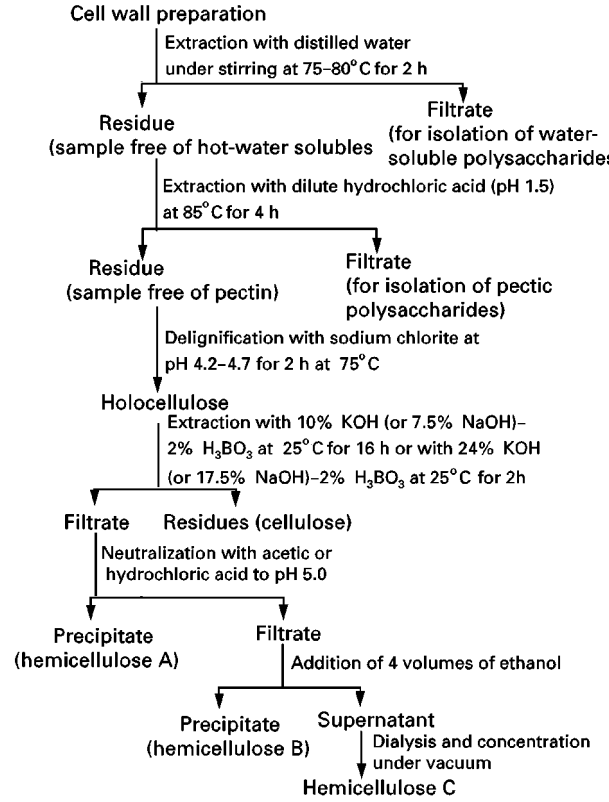
Cell wall preparation
Extraction with distilled water under stirring at 75–80° C for 2 h
Residue
(sample free of hot-water solubles
Filtrate
(for isolation of water-soluble polysaccharides)
Extraction with dilute hydrochloric acid (pH 1.5) at 85° C for 4 h
Residue
(sample free of pectin)
Filtrate
(for isolation of pectic polysaccharides)
Delignification with sodium chlorite at pH 4.2–4.7 for 2 h at 75° C
Holocellulose
Extraction with 10% KOH (or 7.5% NaOH)–2% H3BO3 at 25° C for 16 h or with 24% KOH (or 17.5% NaOH)–2% H3BO3 at 25° C for 2h
Filtrate
Residues (cellulose)
Neutralization with acetic or hydrochloric acid to pH 5.0
Precipitate
(hemicellulose A)
Filtrate
Addition of 4 volumes of ethanol
Precipitate
(hemicellulose B)
Supernatant
Dialysis and concentration under vacuum
Hemicellulose C

Figure 2 Scheme for fractional extraction of polysaccharides from straw or grass.

tissue such as straw and grass (0.5–1.5% dry matter). The term 'pectins' or 'pectic substances' is associated with acidic polysaccharides consisting of a backbone of mainly (1 → 4)-α-bound D-galacturonic acid residues interrupted by the insertion of α-linked L-rhamnose residues. Other constituent sugars such as L-arabinose, D-galactose, D-xylose, L-fucose, and traces of 2-*O*-methyl-D-xylose and 2-*O*-methyl-L-fucose are attached in the side chains. The pectins can be extracted with dilute acid, e.g. HCl solution at pH 1.5 or with chelating agents such as 0.2% aqueous ammonium oxalate, disodium ethylenediaminetetraacetic acid (EDTA), and sodium hexametaphosphate (SHP) solution at pH 3–4 for 4 h at 85°C. After extraction, the filtrates are adjusted to pH 4.0 with 1 M NaOH. The pectic polysaccharides are isolated by precipitation of the filtrates in 4 volumes of 95% ethanol and recovered by centrifugation. Crude pectins are extensively washed with 70% acidified ethanol and fractionated by ion exchange chromatography on a column (550 mm × 15 mm) of DEAE Sepharose Fast Flow (Pharmacia, Sweden), initially equilibrated in 0.005 M NaAc–buffer pH 5.0 or on a QAE Sephadex A-25 column (80 × 1.5 cm i.d.; Pharmacia, Sweden) equilibrated with 10 mM imidazole-HCl buffer (pH 7.0). Other columns used to fractionate the pectic polysaccharides include a DEAE-Sepharose CL6B column (23 × 5 cm; Pharmacia, Sweden) equilibrated with 0.005 M sodium succinate buffer at pH 4.8, and a DEAE-Sephadex A-50 column (20 × 2 cm; Pharmacia, Sweden) previously equilibrated with 10 mM potassium phosphate buffer (pH 6.0). The neutral and acidic pectic polymers are hydrolysed with 2 M trifluoroacetic acid at 120°C for 2 h in sealed ampoules or with pectinase. In comparison, treatment by pectinase has a significant effect on the hydrolysis of the pectic polymers, while the reverse trend is observed during the treatment of the neutral pectic polysaccharides by acid hydrolysis, since galacturonic acid residues in polysaccharides are known to be resistant to acid hydrolysis. For example, endo-1,4-α-polygalacturonase, pectin and pectate lyases can substantially cleave the α-(1 → 4)-GalpA glycosidic bonds between contiguous galacturonic residues. Previous studies have shown that extraction of the cell wall preparation of sugar beet pulp with water followed by treatment with HCl at pH 1.5, 0.2% ammonium oxalate, 0.2% EDTA, and 0.2% SHP at pH 3.3 for 4 h at 85°C yielded 32.1%, 26.1%, 29.3%, and 30.0% (percent dry cell wall preparation) of pectic polysaccharides, respectively. An optimum extraction procedure was found to be treatment with dilute HCl at pH 1.5 for 4 h at 85°C, in which the pectin contained 84.6% anhydrogalacturonic acid and only 2.7% ash. Xylose-rich pectic polysaccharides (1.1% of dry matter) can be obtained from wheat straw by extraction with dilute HCl at pH 1.6 for 4 h at 85°C, which contain 44.8% galacturonic acid (released by pectolyase), and 28.4% neutral sugars (released by acid hydrolysis).

Hemicelluloses

The cell wall components that are readily hydrolysed by hot dilute mineral acids, or dissolved by hot dilute alkalis or cold 5% sodium hydroxide solutions have been termed 'hemicelluloses'. Hemicelluloses belong to a group of heterogeneous polysaccharides which are formed through biosynthetic routes different to the glucose–UDP route of cellulose (a homopolysaccharide). Hemicelluloses of Gramineae such as cereal straws have a backbone of (1 → 4)-linked *β*-D-xylpyranosyl units. The chain may be linear, but is often branched and usually has other glycosidically bound sugar units. Some xylan chains have D-glucopyranosyluronic acid units attached, but the most important acidic hemicelluloses are *O*-acetyl-4-*O*-methyl-D-glucuronoxylans and L-arabino(4-*O*-methyl-D-glucurono)xylans. In dicots, where the hemicelluloses are mostly xyloglucan, hydroxyproline-rich glycoproteins also comprise a substantial amount of the cell wall and cross-link the carbohydrate polymers to form a rigid matrix. In cereal straws of grasses, these proteins have been replaced by esterified and etherified phenolic compounds. The xylans present in the hemicellulosic backbone can be substituted by arabinose, galactose, glucuronic acid, and methyl glucuronic acid. The arabinoxylan can be isolated directly from fully lignified straws or grasses by extraction with aqueous potassium hydroxide or sodium hydroxide. Usually 80–95% of the total xylan present in straw and grass contains a relatively high percentage of associated lignin (5–10%). This high lignin percentage considerably darkens the polysaccharide limiting their industrial application.

Xylans undergo only partial hydrolysis in alkaline solution at room temperature under an atmosphere of nitrogen. The product obtained – except for the fact that acetyl-, feruloyl-, and *p*-coumaroyl appendices have been partially or completely saponified – is quite similar to the native polysaccharide. The hemicelluloses which have a light brown colour contain a relatively small amount of bound lignin (1–2%), and can be quantitatively isolated from the holocellulose by extraction with aqueous alkali. Delignification of the depectinated cell wall preparations obtained from straws or grasses has been performed with sodium chlorite at 75°C for 2 h in acidic solution

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(pH 4.2–4.7) adjusted by 10% acetic acid. The acetylated xylans are soluble in water and in solvents such as dimethylsulfoxide (DMSO), formamide, and *N*,*N*-dimethylformamide. Although only a part of the xylan can be extracted, the advantage is that no chemical changes take place. Aqueous solutions of potassium and sodium hydroxide are mostly used as the alkaline solvent of choice for the extraction of hemicelluloses. The preferred hydroxide is potassium, mainly because the subsequent potassium acetate formed during the neutralization is more soluble in the alcohol used for precipitation than is sodium acetate. Comparison of the extraction ability of 1 M solutions of potassium, sodium, and lithium hydroxide on wheat straw, shows an approximately equal effect in the rate and yield of solubilization of hemicellulose. However, it has been found that sodium hydroxide and lithium hydroxide are more powerful than potassium hydroxide in removing hemicelluloses, especially mannans from wood samples. The yield of hemicelluloses obtained using calcium hydroxide and liquid ammonia was markedly lower than for the respective alkali metal hydroxide solutions. Liquid ammonia, in general, can be used for pre-swelling prior to an alkaline extraction. Moreover, the yield of hemicelluloses strongly depends on a number of important factors, e.g. type of alkali, concentration, temperature and time of extraction. Addition of sodium borate to the alkali facilitates the dissolution of galactoglucomannans and glucomannans. However, any ester groups present are simultaneously saponified during the alkali extractions.

Hemicelluloses obtained by alkali can be subfractionated into hemicellulose A, B, and C. Hemicellulose A, the more linear and less acidic fraction, is isolated from the supernatant by acidifying to pH 5.0 with acetic acid followed by centrifugation. Hemicellulose B, the more acidic or branched fraction, is obtained from the mother liquor by precipitation with 4 volumes of 95% ethanol, then filtered and washed with 70% ethanol. The resultant solid is redissolved in water (after the residual salts were dialysed against water until free from salts), and the hemicellulose B recovered by evaporation under reduced pressure at 45°C or by lyophilization. The fraction that remains soluble in aqueous ethanol is named 'hemicellulosic fraction C' and is isolated by dialysis with water and ethanol until free from salts (Figure 2). The yield and neutral sugar composition as well as content of uronic acids of hemicellulosic subfractions of DMSO-solubles, A, B, and C extracted sequentially by DMSO at 80°C for 2 h and 10% KOH–2% H_3BO_3 at 25°C for 16 h from wheat straw holocellulose, are given in **Table 2**.

Precipitation of the polysaccharide fractions by addition of miscible organic solvents to aqueous solutions is one of the main methods of recovery and purification. Ethanol is the solvent most commonly used, but methanol, acetone, and other organic solvents have also been applied for fractionation of hemicelluloses. In ethanol–water (80 : 20, v/v) the major portion of the polysaccharides are precipitated and only a small amount of short-chain material is left in solution. In addition to the neutral organic solvents, some more specific precipitation agents are also known, e.g. barium hydroxide for glucomannans and cetyltrimethylammonium bromide or hydroxide for glucuronoxylans. Fehling's solution or other copper salts can be used for precipitation of both glucomannans and glucuronoxylans. Hemicellulosic complexes precipitated by iodine in calcium chloride appear to be relatively unsubstituted by non-xylose residues, whereas the material remaining in solution is more highly substituted by such residues. The methods used to fractionate the hemicelluloses of straws and grasses are similar to those used to fractionate hemicelluloses from woods. The hemicelluloses may be fractionated as their acetates by precipitation from solution by ammonium sulfate, or by chromatography on DEAE-cellulose. The

Table 2 The yield and neutral sugar composition as well as content of uronic acids of hemicellulosic subfractions of DMSO-solubles, A, B, and C extracted sequentially by DMSO at 80°C for 2 h and 10% KOH–2% H_3BO_3 at 25°C for 16 h from wheat straw holocellulose

Hemicellulosic subfractions	*Yield (%)**	*Neutral sugar composition (%)***					*Uronic acids (%)***	*Acetyl content (%)***
		Ara	*Xyl*	*Man*	*Gal*	*Glc*		
DMSO-solubles	4.8	8.5	68.1	Trace	3.8	6.5	5.2	7.3
A	7.2	5.2	86.3	0.4	2.4	2.4	2.6	ND***
B	18.5	11.0	70.0	1.2	5.0	5.3	6.8	ND
C	2.7	12.2	80.3	0.5	3.5	2.0	0.6	ND

*Percent dry matter (w/w).

**Percent hemicellulosic subfractions (w/w).

***ND = not detectable.

precipitated hemicellulose preparations can if desired be further purified by column chromatography.

Gel permeation chromatography is one of the most useful tools for determining the average molecular weights of the isolated hemicellulosic fractions. Ion exchangers based on cellulose, dextran or agarose such as diethylaminoethyl cellulose in different ionic forms can also be used to separate hemicelluloses from each other. Chromatography (in its various forms) is routinely used for the characterization of the acidic hydrolysis products of isolated hemicelluloses. Dialysis of aqueous solutions often removes inorganic salts and other low-molecular-weight impurities prior to chromatography. Alternatively, salt may be removed by electrodialysis, by treatment of solutions with ion-exchange resins, or by gel filtration, using Sephadex, a cross-linked dextran column.

More recently, it has been reported that alkaline peroxide is an effective agent for both delignification and solubilization of hemicelluloses from straws and grasses. This was first proposed in 1984 in studies on the alkaline peroxide delignification of agricultural residues to enhance enzymatic saccharification. Hydrogen peroxide is widely used in the pulp and paper industry to bleach lignin-rich pulps. It has been generally accepted that the bleaching action of hydrogen peroxide is attributable to the hydroperoxide ion (HOO^-), formed in alkaline media, which is the principal active species in hydrogen peroxide bleaching systems. This anion is a strong nucleophile that preferentially attacks ethylenic and carbonyl groups present in lignin. On the other hand, hydrogen peroxide is unstable in alkaline conditions and readily decomposes, particularly in the presence of certain transition metals such as manganese, iron, and copper. This metal-catalysed decomposition of hydrogen peroxide is undesirable in the bleaching operation. However, this decomposition generates more active radicals, such as hydroxyl radicals ($HO^\bullet$) and superoxide anion radicals ($O_2^{-\bullet}$), participating in degradation reaction of lignin and solubilization of hemicelluloses, which therefore, results in significant solubility of the lignin and hemicelluloses. The advantages of hemicellulose extraction with alkaline peroxides are low investment cost, accompanying strong bleaching effect, lower biological and chemical oxygen demand (BOD and COD) effluents as well as the recovery of the solubilized macromolecular hemicelluloses with a minimal degradation. Results show that more than 80% of the original hemicelluloses and over 90% of the original lignin is solubilized during the treatment of cereal straws such as wheat, barley, rice, oat, and rye straw, and maize stems with 2% H_2O_2 at 48°C for 16 h at pH 12.0–12.5 (**Table 3**). These hemicellulose preparations are white in colour and contain very small amounts of associated lignin (3–5%).

To gain maximum dissolution of the hemicelluloses, it is not necessary to continuously regulate the reaction pH, even though over the course of the treatment (48°C, 16 h) the reaction pH rises from 12.0 to 12.5 and from 12.9 to 13.1, respectively. As the reaction pH becomes more alkaline, increasing amounts of hemicelluloses are solubilized, and the yield of the residue decreases. Incremental increase of the initial reaction pH from 11.5 to 12.5 results in an increase of hemicellulose dissolution of about 20%. During the initial stages of stirring, oxygen evolution is active, and substantial frothing occurs, requiring extractions to be conducted in vessels with volumes two to three times those of the extraction mixtures. After treatment, the cellulose-rich insoluble residue is collected by filtration, washed with distilled water until the pH of the filtrate is neutral, and then dried at 60°C. The supernatant fluid is adjusted to pH 5.5 with 10% HCl and then concentrated. The solubilized hemicelluloses are precipitated by pouring the concentrated supernatant into 4 volumes of

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Table 3 The yield, neutral sugar composition, and content of uronic acid and lignin of hemicelluloses obtained by treatment of wheat, rice, rye, barley, and oat straw, and maize stems respectively with 2% H_2O_2 at 48°C for 16 h at pH 12.2

Cereal straw/stems	*Yield (%)**	*Neutral sugar composition (%)***							*Uronic acids (%)***	*Lignin content (%)***
		Rha	*Fuc*	*Ara*	*Xyl*	*Man*	*Glc*	*Gal*		
Wheat	29.6	0.8	ND***	13.8	60.5	0.4	9.8	4.5	4.9	5.1
Rice	22.3	0.6	0.3	14.9	56.3	ND	22.3	4.8	4.3	4.7
Rye	26.6	0.5	ND	11.2	65.3	0.3	6.1	3.3	8.1	4.8
Oat	25.6	0.4	ND	10.8	68.3	0.3	6.4	3.6	5.5	4.3
Barley	23.3	0.4	ND	10.6	66.1	0.5	7.6	3.7	5.8	4.5
Maize	22.7	0.5	0.3	15.2	62.7	0.8	6.3	4.7	5.3	3.7

*Percent dry matter (w/w).
**Percent hemicelluloses (w/w).
***ND = not detectable.

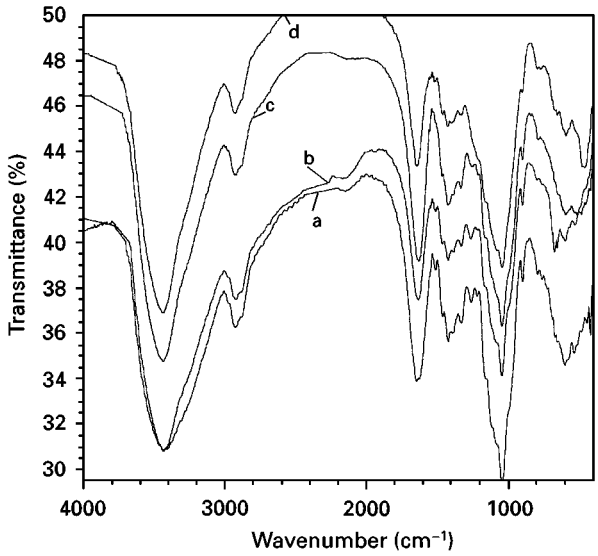

Figure 3 FT-IR spectra of 2% aqueous hydrogen peroxide-soluble hemicellulosic preparations extracted with 2% H_2O_2 at 50°C for 12 h at pH 11.5 from wheat straw (spectrum a), rye straw (spectrum b), maize stem (spectrum c), and rice straw (spectrum d).

ethanol, from which they settle out as a white flocculent precipitate which is freeze-dried. The Fourier transform infrared (FT-IR) spectra of 2% aqueous hydrogen peroxide-soluble hemicellulosic preparations extracted with 2% H_2O_2 at 50°C for 12 h at pH 11.5 are shown in **Figure 3**: wheat straw (spectrum a), rye straw (spectrum b), maize stem (spectrum c), and rice straw (spectrum d). **Figure 4** illustrates the ^{13}C-NMR spectrum of the hemicelluloses extracted with 2% H_2O_2 at 45°C for 12 h at pH 12.0 from maize stems. Obviously, both FT-IR spectra and ^{13}C-NMR spectrum of the hemicelluloses appear to be those of the typical hemicelluloses such as xylan from cereal straws and grasses. These observations reveal

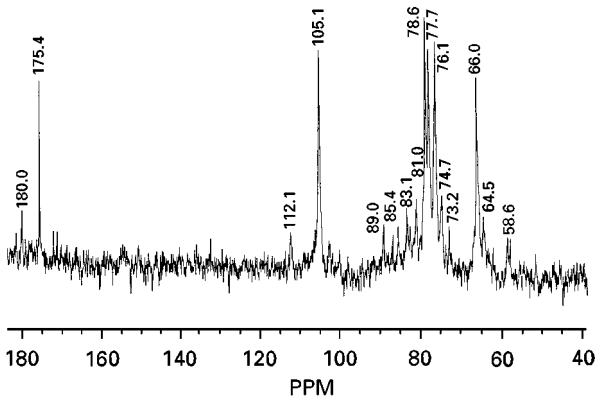

Figure 4 ^{13}C-NMR spectrum of the hemicelluloses extracted with 2% H_2O_2 at 45°C for 12 h at pH 12.0 from maize stems.

that the alkaline peroxide treatments under the conditions given do not affect the overall structure of the macromolecular hemicelluloses. This effective and convenient method of alkaline peroxide treatment may be used for most isolation of hemicelluloses from straws and grasses.

Steam treatment enables the lignocellulosic materials present in straws and grasses to be separated into hemicelluloses, lignin, and cellulose in reasonable yields and purity. Steam-explosion treatments or other steam treatments have the advantage of using a widely-available solvent without significant cost or environmental impact. It is generally agreed that the steaming process is basically an acid-catalysed autohydrolysis due to the small amounts of acetic acid liberated early in the process through cleavage of the hemicellulosic acetyl groups. A portion of the starting material, mainly hemicellulose, is converted into water-soluble products through this acid-catalysed hydrolysis process. The cellulose is not significantly solubilized but undergoes a change in its crystallinity or is partially depolymerized. During this process, the lignocellulosic substrate is stream treated at temperatures ranging from 140 to 240°C for 1–10 min followed by a rapid pressure release (i.e. explosion) through a discharge valve. The hemicelluloses solubilized during the steam or steam-explosion process, are isolated after extraction in water. They comprise mainly acetyl- and 4-O-methylglucuronosyl-substituted xylans, and contain 15–25% and 5–10% bound lignin, respectively. The associated lignins can be removed by treatment of the crude hemicelluloses with 1 M NaOH at 20°C for 2–4 h. All the hemicellulosic preparations have a lower degree of polymerization (40–65), with $\bar{M}_w$ ranging between 5000 and 10 000 g mol^{-1}.

Cellulose

Cellulose is the main constituent of agricultural residues. Approximately 35–45% of the dry substance in most straw and grass species is cellulose, located predominantly in the secondary cell wall. The term 'α-cellulose' is given to the residue remaining after delignification by sodium chlorite in acidic solution and separation of hemicelluloses by extraction of the holocellulose with 24% KOH (or 17.5% NaOH) at 25°C for 2 h or 10% KOH (or 7.5% NaOH) at 25°C for 16 h. This term was originally coined for wood cellulose which is insoluble in strong sodium hydroxide solution. The portion which is soluble in the alkaline medium but precipitated from the neutralized solution was called 'β-cellulose'. 'γ-Cellulose' is the name for the portion which remains soluble even in the neutralized solution. This method was

modified in various ways and is now established as the standard method for the determination of α-, β-, and γ-cellulose from straws and grasses. The cellulose-rich residues remaining after alkaline peroxide treatment under the conditions given earlier contain 80–90% cellulose and 10–20% hemicelluloses as well as approximately 5% bound lignin. During steam treatment, cellulose undergoes a change in its crystallinity and can also partially depolymerize, depending on the treatment conditions. The cellulose-rich fibres generated by the steam treatment/explosion process are generally more accessible to chemicals and enzymes under derivatization conditions.

Further Developments

The current views on the fractional isolation of polysaccharides from cereal straws and grasses are based on the culmination of information gained over the past thirty years. In spite of the many studies on the polysaccharides of straws and grasses, little is known about (a) isolation of pectic polysaccharides, (b) isolation of the polysaccharide fraction present in delignification liquors, and (c) isolation of non-glucosyl residues and residual lignins in α-cellulose. Procedures designed to extract pectic substances may extract material that might otherwise be described as partly hemicellulosic, and the converse may also happen. Many procedures used in the isolation of polysaccharides from straws and grasses would lead to the loss of any such polysaccharides present. During delignification of wheat straw with sodium chlorite in acidified solution, 1.2–2.4% of the total hemicelluloses passes into solution. It is also more difficult to isolate pure cellulose without degradation since the hemicelluloses and lignin are strongly associated with cellulose; many of the solubilized hemicelluloses are irreversibly absorbed on the cellulose. In addition, drying straw and grasses in an oven at 60°C may cause autohydrolysis or other changes in polysaccharide morphology. Furthermore, the various procedures used prior to the exhaustive extraction of hemicelluloses may affect quantitative or structural conclusions about the hemicelluloses as they occur in nature. It is also of interest to note that the hemicellulosic materials extracted by alkali are, only in part, precipitated in ethnol after neutralization of the extract. Under these conditions, a small part of the hemicellulosic materials remains in solution, and is commonly not recovered. All of these points require further investigation to obtain a more reliable standard method for fractional isolation of polysaccharides from cereal straws and grasses.

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